REVIEW ARTICLE

The glucose transporter family: structure, function and tissue-specific expression

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INTRODUCTION

The transport of glucose across the plasma membrane of mammalian cells represents one of the most important cellular nutrient transport events, since glucose plays a central role in cellular homeostasis and metabolism. It has long been established that the plasma membranes of virtually all mammalian cells possess a transport system for glucose of the facilitative diffusion type; these transporters allow the movement of glucose across the plasma membrane down its chemical gradient either into or out of cells. These transporters are specific for the D-enantiomer of glucose and are not coupled to any energy-requiring components, such as ATP hydrolysis or a H^+ gradient [1]. The facilitative glucose transporters are distinct from the Na^+-dependent transporters, which actively accumulate glucose [2,3].

The importance of glucose as a cellular metabolite has led to a great deal of research into the mechanism of this transport event. However, the realization that glucose transport into certain tissues of higher mammals is under both acute and chronic control by circulating hormones, and that defects in this transport system may underlie diseases such as diabetes mellitus, has led to an almost exponential growth in research effort in the transporter field over the past 5–10 years. Perhaps the most significant observation to arise during this time is the realization that, rather than being mediated by a single transporter expressed in all tissues, glucose transport is mediated by a family of highly related transporters which are the products of distinct genes and are expressed in a highly controlled tissue-specific fashion [2] (Table 1). The development and maintenance of this genetic diversity clearly implies a teleological requirement for multiple glucose transport proteins expressed in different tissues, with each being likely to play a distinct role in the regulation of whole-body glucose homeostasis. Some clues as to the relationship between the tissue-specific patterns of expression and the different kinetic characteristics of each of these transporters have recently been provided from an examination of the properties of the isolated transporters in expression systems such as the *Xenopus* oocyte system.

In this review we summarize the present state of knowledge of the currently identified members of the glucose transporter family, propose a basis for their diversity highlighting differences in kinetic properties, substrate specificity and hormonal regulation, and discuss aberrant expression and/or dysfunction of these transporters in disease states.

THE TRANSPORTER FAMILY

GLUT 1: the erythrocyte-type glucose transporter

Perhaps the best-studied glucose transporter is that present in human red blood cell membranes. Erythrocytes provide a rich source of this transporter, with it comprising about 3–5% of the membrane protein. The isolation of this protein by Lienhard and his co-workers in the early 1980s represented a major advance in the study of glucose transport [4]. The purified protein enabled a study of the kinetics of the transport system in defined lipid environments and also led to the generation of antibody probes [5–8]. These antibodies, together with partial sequence information from the protein, resulted in the isolation of a cDNA clone for the transporter in 1985 [9,10]. The gene encoding this transporter has also been isolated [11,12].

Utilizing both cDNA and antibody probes, many subsequent studies have demonstrated that both the GLUT 1 protein and its mRNA are present in many tissues and cells [13,14]. It is expressed at highest levels in brain but is also enriched in the cells of the blood–tissue barriers such as the blood–brain/nerve barrier, the placenta, the retina, etc. [15]. In addition, the GLUT 1 protein has been identified in muscle and fat, tissues which exhibit acute insulin-stimulated glucose transport, but only at very low levels in the liver, the other major tissue involved in whole-body glucose homeostasis [13].

It is well established that transformation of cell culture lines results in a pronounced elevation of GLUT 1 protein and mRNA levels, and that this general phenomenon is observed for all cell culture lines [16–21]. Moreover, it is clear that many, if not all, mitogens stimulate GLUT 1 transcription, and that glucose starvation can also stimulate GLUT 1 expression [22–27]. One potential advantage to the cell of increasing GLUT 1 may be related to the kinetic asymmetry property of this isoform [28,29]. The net influx K_m for glucose by GLUT 1 is 1.6 mM, significantly lower than either the equilibrium-exchange or net efflux K_m values (see Table 2 for a description of differences between net and exchange fluxes). The kinetic asymmetry of GLUT 1 appears to be allosterically regulated by binding of intracellular metabolites and is inhibited by intracellular ATP [28]. We would propose that this asymmetry would allow this transporter to function effectively as a unidirectional transporter under conditions where extracellular glucose is low and the intracellular demand for glucose is high, such as would occur during glucose starvation of cells in culture.

GLUT 2: the liver-type glucose transporter

The ability to detect only very low levels of GLUT 1 in hepatocyte membranes, coupled to the observation that the kinetics of glucose transport in hepatocytes were radically different from those in erythrocytes, led to the proposal that a distinct transporter may be expressed in hepatocytes [30,31]. To identify this transporter two laboratories independently developed a tech-

Abbreviations used: 3-*O*-MG, 3-*O*-methyl-D-glucose; IAPS-forskolin, 3-iodo-4-azidophenethylamido-7-*O*-succinyl-forskolin; ATB-BMPA, 2-*N*-4-(1-azi-2,2,2-trifluoroethyl)-benzoyl-1,3-bis-(D-mannos-4-yloxy)-2-propylamine.

Table 1 Major sites of expression of the different glucose transporters

Isoform	Tissue	References
GLUT 1	Placenta; brain; blood–tissue barrier; adipose and muscle tissue (low levels); tissue culture cells; transformed cells	9,10,13,15,17,18
GLUT 2	Liver; pancreatic β-cell; kidney proximal tubule and small intestine (basolateral membranes)	32–35
GLUT 3	Brain and nerve cells in rodents; brain, nerve; low levels in placenta, kidney, liver and heart (humans)	37–40
GLUT 4	Muscle, heart and adipose tissue	42–46,96–98
GLUT 5	Small intestine (apical membranes); brain, muscle and adipose tissue (muscle and brain at low levels)	60,61,64
GLUT 7	Microsomal glucose transporter; liver	65

Table 2 Kinetic parameters of the glucose transporter family expressed in *Xenopus* oocytes

A variety of different steady-state approaches can be used to determine kinetic constants for glucose transporters. These assays all measure the rate of glucose transport across the membrane, but under different conditions. It should be noted that GLUT 1, but not GLUTs 2 or 4, are asymmetrical with regard to the interactions of glucose at the two sides of the membrane. Note also that K_m and $V_{max.}$ need not be the same when measured under these different conditions because the re-orientation of the binding site may be faster in the presence of unlabelled sugar (the equilibrium-exchange experiment). (i) Equilibrium-exchange transport: the same concentration of sugar is present on both sides of the membrane, but the radioactive label is present only on one side. In exchange-influx experiments, the transporter can return to the outward-facing conformation with unlabelled sugar bound. (ii) Zero-trans transport: sugar is present only on one side of the membrane. In net influx experiments, the transporter will return to the outward-facing conformation unoccupied at initial time points when the intracellular sugar concentration is low. Values are from references [2], [36], [41], [57], [63] and [160].

	K_m (mM)			
Isoform	3-*O*-MG (equilibrium exchange)	2-Deoxyglucose (net influx)	Asymmetrical?	Other transported substrates
GLUT 1	20.9 ± 2.9	6.9 ± 1.5	Yes	Galactose ($K_m \sim 17$ mM)
GLUT 2	42.3 ± 4.1	11.2 ± 1.1	No	Fructose ($K_m \sim 66$ mM)
GLUT 3	10.6 ± 1.3	1.4 ± 0.06	Not known	Galactose ($K_m \sim 8.5$ mM)
GLUT 4	1.8	4.6 ± 0.3	No	Not studied in oocytes
GLUT 5	n.d.	n.d.	Not known	Fructose ($K_m \sim 6$ mM)

nique for isolating transporter-like cDNAs from additional tissues/cells, including liver. The approach developed involved the use of the GLUT 1 cDNA to probe libraries from hepatocytes under conditions of low stringency, with the rationale that only cDNAs which were similar to GLUT 1 would be identified. Using this approach, Thorens et al. [32] and Fukumoto et al. [33] were able to isolate a cDNA from hepatocytes which, upon analysis of the predicted amino acid sequence, proved to exhibit a high degree of homology to GLUT 1. Furthermore, hydropathy plots of the GLUT 1 and GLUT 2 proteins are virtually superimposable, suggesting that the two transporters are likely to adopt similar global shapes within the membrane.

Subsequent analysis of the sites of expression of GLUT 2 demonstrated that this isoform is expressed at highest levels in the liver, pancreatic β-cell (but not the α- or δ-cells), and on the basolateral surface of kidney and small-intestine epithelia [34,35]. Analysis of the equilibrium-exchange K_m of this isoform for a glucose analogue, 3-*O*-methyl-D-glucose (3-*O*-MG), when expressed in *Xenopus* oocytes revealed that this transporter exhibited a supraphysiological K_m for 3-*O*-MG for GLUT 2 of 42 mM [36]. This high K_m value for GLUT 2 is in agreement with data published for intact hepatocytes, where a K_m for glucose of approx. 66 mM has been reported [30]. The presence of a high-capacity high-K_m transporter in hepatocytes is therefore adventitious for rapid glucose efflux following gluconeogenesis.

This high K_m value may provide a rationale for GLUT 2 localization to those tissues that are involved in the net release of glucose during fasting (liver), glucose sensing (β-cells) and transepithelial transport of glucose (kidney and small intestine), since glucose flux through this transporter at physiological glucose concentrations would be predicted to change in a virtually linear fashion with extracellular/intracellular glucose concentration. This would result in the highly favourable condition that transporter saturation by glucose would not be rate limiting.

Perhaps the more important functional consequence of the presence of GLUT 2 in kidney and intestinal epithelial cells is its high transport capacity compared with the other transporters. Glucose transport in both the intestine and the kidney is a two-step process, with the active accumulation of glucose via a Na^+-dependent transporter on the apical membrane of the small intestine transporting glucose against its concentration gradient [3]. The accumulated glucose is subsequently released into the capillaries via the high-capacity GLUT 2 which is present at the basolateral borders.

GLUT 3: the brain-type glucose transporter

The development of the low-stringency hybridization approach to cloning glucose transporter cDNAs was subsequently applied to other tissues. In an effort to identify the transporter species present in skeletal muscle, Bell and his co-workers screened a human fetal muscle library for the expression of transporter-like proteins. A novel transporter-like cDNA, GLUT 3, was isolated using this approach [37,38]. Surprisingly, Northern blot analysis revealed that this transporter was barely detectable in adult skeletal muscle, its predominant site of expression being the brain, with lower levels in fat, kidney, liver and muscle tissue. Anti-peptide antibodies specific for either the mouse or human

isoforms of GLUT 3 have been used in an effort to further evaluate the role of GLUT 3 in these tissues [39,40]. Using the anti-(mouse GLUT 3) antibodies it has been demonstrated that the expression of GLUT 3 is restricted to brain and neural cell lines and is not immunologically detectable in highly purified mouse muscle, liver or fat membranes. Immunological analysis of human tissues revealed the presence of GLUT 3 at high levels in the brain, with lower amounts present in the placenta, liver, heart and kidney, but not in three different muscle groups, i.e. soleus, vastus lateralis and psoas major [40]. These latter results appear to be in discordance with the relative abundance of GLUT 3 mRNAs in these tissues: for example, the mRNA levels of GLUT 3 in kidney and placenta appear to be roughly 50% of that recorded in brain, but in contrast the level of GLUT 3 protein in these tissues is much lower. One explanation for the disparity between Northern and immunoblot levels of GLUT 3 could be the presence of significant neural contamination of the tissue sections used to prepare the mRNA for the Northern analysis. Alternatively, these tissues may exhibit a negative post-transcriptional regulation of this species of transporter.

Thus it appears that high GLUT 3 protein expression levels are confined generally only to tissues which exhibit a high glucose demand (brain, nerve). Therefore, this isoform may be specialized to act in tandem with GLUT 1 to meet the high energy demands of such tissues. The low level of apparent expression of GLUT 3 protein in liver and kidney may be the result of the localization of this isoform to a specific subset of cells within these tissues.

GLUT 3 exhibits a K_m for 3-*O*-MG exchange transport of about 10 mM [36,41]. It is well established that the major glucose transporter expressed at the blood–nerve and blood–brain barrier is GLUT 1, which has a higher equilibrium-exchange K_m than GLUT 3. In brain, under normal conditions the capacity of hexokinase for glucose (the preferred energy source) is considerably greater than the capacity of the glucose transport systems in this tissue. However, under conditions of either high glucose demand or hypoglycaemia, the expression of GLUT 3 in the brain with a low K_m for hexoses may be required to successfully utilize low concentrations of blood glucose.

GLUT 4: the insulin-responsive glucose transporter

Following the success in utilizing a GLUT 1 cDNA probe to obtain the homologous sequences of GLUT 2 and GLUT 3, there was enormous excitement in many laboratories as it was realized that the unique glucose transport regulation found in insulin-responsive fat and muscle tissue could be due to a fourth isoform. This was followed by feverish activity by several independent groups and there appeared in 1989 five separate reports of the cloning and sequencing of the GLUT 4 isoform [42–46]. This isoform was shown to occur only in muscle and adipose tissue.

In rat adipose cells, insulin produces an approximate 20–30-fold increase in glucose transport [47–51]. In human adipose cells the response to insulin is much smaller, approximately 2–4-fold [52]. Insulin has been shown to increase glucose transport activity in rat muscle by 7-fold [53] but only by 2-fold in human muscle [54]. Kinetic studies [47–50] have shown that the major effect of insulin is to increase the $V_{max.}$ of glucose uptake. Small changes in the K_m have been reported in rat adipose cells [55] and 3T3-L1 cells [56] but these differences are probably related to the greater proportion of GLUT 1 in the plasma membrane of non-insulin-treated cells. Several potential mechanisms for the increase in $V_{max.}$ can be considered. The increase in $V_{max.}$ for glucose uptake could occur if insulin increased the intrinsic activity of the transporter (i.e. the catalytic rate constant of each transporter present in the membrane). Another related, and potentially plausible, mechanism would be an insulin-induced conformational redistribution of transport sites between the outside and inside surfaces of the plasma membrane in an asymmetrical transporter. Such a transporter would exhibit differences in K_m at the inner and outer surfaces and a difference between net and exchange flux as occurs in GLUT 1. However, studies investigating this mechanism have shown that the adipocyte transporter, now known to be GLUT 4, has kinetically symmetrical affinities for 3-*O*-MG influx and efflux and does not show accelerated exchange [47,49]. Another potential mechanism, and the one most supported by recent evidence, suggests that the majority of the acute insulin-stimulated increase in glucose transport measured in adipocytes and muscle is mediated by the appearance of additional GLUT 4 in the plasma membrane. The insulin regulation of GLUT 4 translocation is discussed later. There has been much debate concerning whether translocation can account for the full extent of glucose transport stimulation by insulin. Czech's group in particular have suggested that insulin induces changes in the intrinsic catalytic activity of transporters, possibly mediated by conformational redistribution of sites locked in an inwardly directed conformation due to the binding of an allosteric transport regulator [112]. While it is difficult to reconcile this hypothesis with the observation of kinetic symmetry of GLUT 4, there are precedents for such a mechanism, as GLUT 1 can exhibit allosteric regulation of asymmetry (see above) and mutations of GLUT 1 cause conformational locking into an inwardly directed conformation (see below). However, we consider that intrinsic activation is unlikely to be a major mechanism for GLUT 4 regulation by insulin and that the apparent discrepancies between the observed extent of translocation and the level of transport stimulation may be partly due to the presence of precursor intermediate states in the GLUT 4 trafficking pathway. In addition, many of the discrepancies between the level of transport stimulation induced by insulin and the fold change in GLUT 4 as detected by Western blotting of plasma membrane fractions are due to the difficulties inherent in obtaining highly purified plasma membrane fractions.

The most important property of GLUT 4, which distinguishes it from other isoforms, is its propensity to remain localized in intracellular vesicles in the absence of insulin. Insulin can then specifically recruit this transporter to the surface under metabolically appropriate conditions. The relatively low K_m value of this transporter (2–5 mM) [47–49,57,58] would ensure that it operates close to its $V_{max.}$ over the normal range of blood glucose concentrations, and this ensures the rapid removal of blood glucose into the body's energy stores of glycogen and triacylglycerol. In the absence of insulin (the basal state), glucose transport is rate limiting for metabolism, but insulin stimulates an increase in the plasma membrane abundance of GLUT 4 transporters so that insulin-stimulated transport does not limit metabolism [59].

GLUT 5: the small-intestine sugar transporter

Hexose transport/absorption in the small intestine is clearly an important aspect of whole-body glucose homeostasis. Bell and colleagues in his laboratory isolated another putative glucose transporter cDNA from human small intestine [60]. Northern blot analysis has suggested that this isoform is present at high levels in small intestine. Similar results have been obtained using specific anti-peptide antibodies; moreover, the protein appears to be localized exclusively to the apical brush border on the

luminal side of the epithelial cells [61]. Since the transport of glucose from the lumen into the epithelial cells is, under normal circumstances, mediated predominantly by the unrelated Na^+-dependent glucose transporter [62], the presence of a putative facilitative glucose transporter in the brush border is not easily explained. The explanation for the presence of GLUT 5 in the brush border has been provided by the recent demonstration that GLUT 5 is a high-affinity fructose transporter, with an apparently poor ability to transport glucose [63]. Thus, on the luminal surface of the small intestine, the primary role of GLUT 5 would be the uptake of dietary fructose.

Northern and immuno-blot analyses have recently demonstrated that this protein is expressed in a range of tissues, including muscle (soleus, rectus abdominus, psoas major and vastus lateralis), brain and adipose tissue. This can be rationalized if GLUT 5 functions to supply these tissues with fructose. However, it is not clear if other fructose transporters also exist. It appears that, unlike GLUT 4, this transporter does not undergo insulin-stimulated translocation in adipocytes, consistent with an apparent lack of insulin-stimulated fructose transport in human adipocytes [64].

GLUT 6: a pseudogene-like sequence

The homology screening approach used by Bell and his colleagues has identified a further transporter-like transcript, with an apparently ubiquitous tissue distribution [60]. Sequence analysis of a cDNA clone for this transcript revealed a high level of base identity (79.6%) with GLUT 3. However, the cDNA was found to contain multiple stop codons and frame shifts, and is unlikely to encode a functional glucose transporter [60]. The extensive identity of the GLUT 6 cDNA with the GLUT 3 cDNA sequence suggests that the glucose transporter-like region of the GLUT 6 transcript may have arisen by the insertion of a reverse-transcribed copy of GLUT 3 into the non-coding region of a ubiquitously expressed gene [60].

GLUT 7: the hepatic microsomal glucose transporter

In the liver, glucose is produced from gluconeogenesis and glycogenolysis for export into the blood. The terminal step of both these processes is the removal of phosphate from glucose 6-phosphate by a specific phosphatase. Glucose-6-phosphatase is a multicomponent enzyme, and it is well established that the glucose produced as a result of the action of this phosphatase is initially confined to the lumen of the endoplasmic reticulum. Thus, in order for the glucose produced to be exported from the liver, it must first cross the endoplasmic reticulum membrane. The work of Burchell and her colleagues has recently revealed that the mechanism by which glucose crosses the endoplasmic reticulum membrane is via a unique member of the facilitated-diffusion-type transporters, now called GLUT 7 [65].

This latest member of the transporter family has been demonstrated to exhibit a close relationship to GLUT 2, there being 68% identity at the amino acid level. One important difference between GLUTs 2 and 7 is the presence of a unique sequence of six amino acids at the C-terminus of GLUT 7. These six amino acids contain a consensus motif for the retention of membrane-spanning proteins in the endoplasmic reticulum (KKMKND). Interestingly, the GLUT 7 protein is virtually identical with GLUT 2 throughout the first four membrane-spanning domains, and also in the regions of transmembrane helices 9 and 10. Moreover, the cDNA sequence is 100% identical with that of GLUT 2 at three locations. Surprisingly, these regions of identity do not coincide with the intron–exon boundaries [2], suggesting that GLUT 7 is unlikely to be a simple splice variant. However, the lack of base-drift in the third position of the codons over significant stretches of the cDNA raises the intriguing possibility of a unique and complex splicing mechanism generating GLUTs 2 and 7 [65].

GLUCOSE TRANSPORTER STRUCTURE

Analysis of the predicted amino acid sequences of the mammalian glucose transporters shows that these are highly homologous with one another. The mammalian transporters possess high levels of sequence identity with transporters found in many species including cyanobacteria [66], *Escherichia coli* [67], *Zymomonas mobilis* [68], yeast [69,70], algae [71], protozoa [72,73] and plants [74]. This high level of sequence similarity is probably related both to a common mechanism of transport catalysis and also to the transport of a common type of substrate. There are, however, extremes to this generalization and the family includes transporters which differ in some aspects of mechanism, from those which are purely facilitative diffusion types in mammals to the H^+-coupled symporters that occur in bacteria [67]. Similarly, the range of preferred substrates includes hexoses, pentoses [67] and disaccharides [70]. Interestingly, the family of homologous proteins includes two transporters that transport the non-sugar substrate quinnate (a hydroxylated six-membered ring substrate) [75].

The common features revealed by sequence alignment and analysis of all the above-mentioned transporters include 12 predicted amphipathic helices arranged so that both the N- and C-termini are at the cytoplasmic surface (Figure 1). There are large loops between helices 1 and 2 and between helices 6 and 7. The large loop between helices 6 and 7 divides the structure into two halves, the N-terminal domain and the C-terminal domain. The loops between the remainder of the helices at the cytoplasmic surface are very short and the length of these loops (about eight residues) is a conserved feature of the whole family. These short loops place severe constraints on the possible tertiary structures and suggest very close packing of the helices at the inner surface of the membrane in each half of the protein. The length and sequence identity of the loops at the extracellular surface of these proteins are very varied but are generally longer than the loops at the cytoplasmic surface. This may potentially result in a less compact helical packing at the external surface. The two-dimensional topography with N- and C-termini on the cytoplasmic surface (Figure 1) has been confirmed using anti-peptide antibodies which react only when the inner surface of the transporter is exposed, as in inverted vesicles containing human erythrocyte GLUT 1. Infra-red spectroscopy has suggested a high (over 80%) helical content for the GLUT 1 protein [76,77].

Conserved motifs in the glucose transporters include GRR(K) between helices 1 and 2 in the N-terminal half, and correspondingly between helices 7 and 8 in the C-terminal half. Similarly, EXXXXXXR occurs between helices 4 and 5 in the N-terminal half and correspondingly between helices 10 and 11 in the C-terminal half. These motifs may be conserved to maintain conformational stability of the protein and may be involved in salt-bridging between helices. The repetition of these motifs between the two halves of the protein suggests that duplication of a gene encoding an ancestral six-membrane-spanning helical protein may have produced the two-domain 12-membrane-spanning helical structure that is so highly conserved in the sugar transporter family. The constraints imposed by the short cytoplasmic loops suggest that a single group of 12 helices is unlikely, but instead the six helices in each of the N- and C-terminal

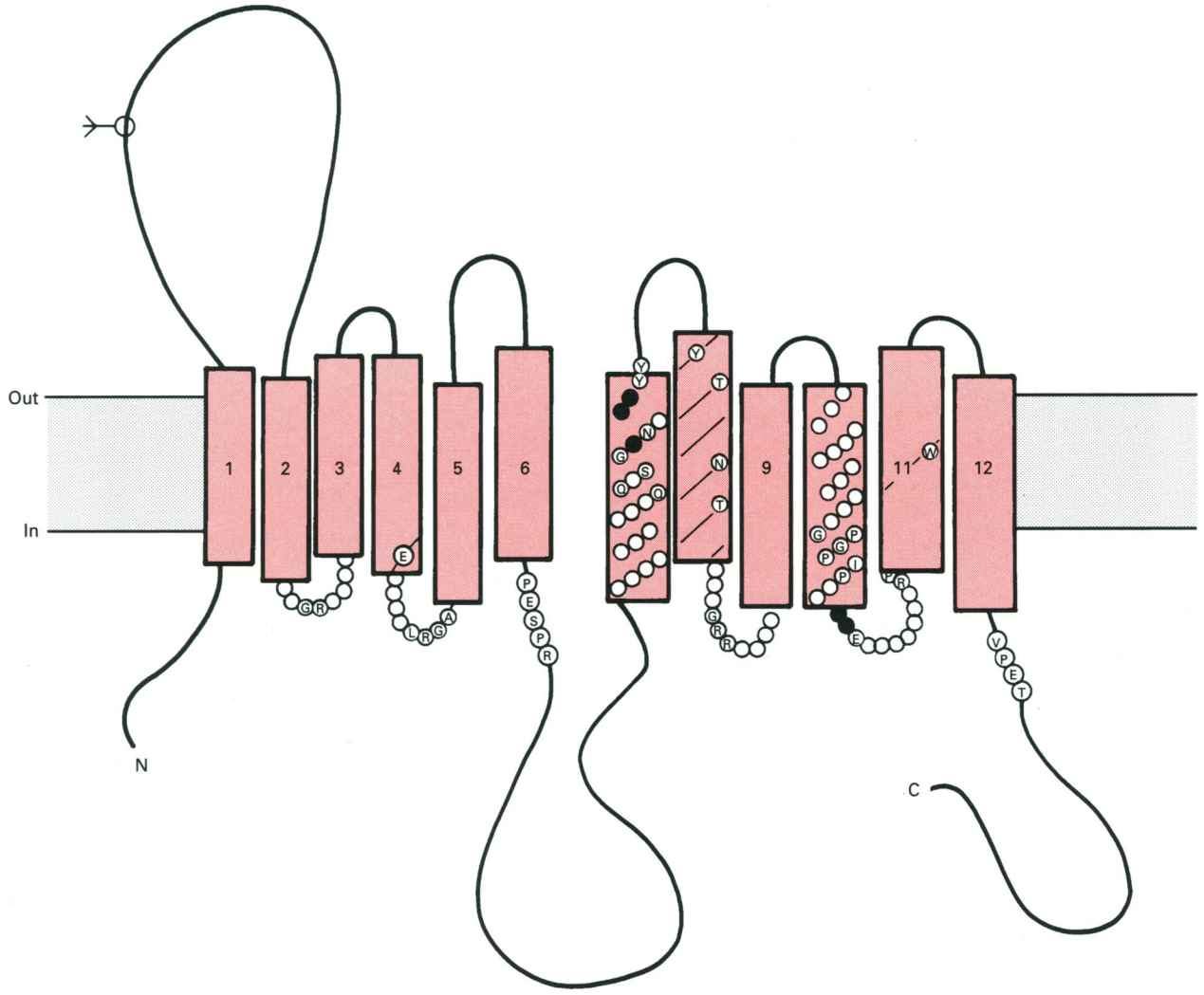

Figure 1 Hypothetical model for the structure of the glucose transporters

The protein is predicted to contain 12 transmembrane helices (1–12), with both the N- and C-termini intracellularly disposed. N-linked glycosylation can occur in the extracellular loop between helices 1 and 2 as shown. Conserved amino acids are indicated by the appropriate single-letter code; filled circles indicate conservative substitutions. Note that not all conserved amino acids are shown (see the text).

domains may be separately closely packed to produce a bilobular structure similar to that which has been observed in low-resolution electron microscopic images of the *E. coli* lactose permease [78]. This packing arrangement has been incorporated into a molecular model of the hexose transporter GLUT 1 [81] (Figure 2).

Molecular modelling suggests that most of the highly conserved residues in helical regions occur on the faces of helices that are directed to the centre of the protein and away from the membrane lipid. Conserved regions of particular interest occur in the C-terminal half of the protein and may be involved in ligand recognition. The motif QQXSGXNXXXYY in helix 7 is present in all the mammalian transporters and is highly conserved in all members of the wider glucose transporter superfamily. The first glutamine (Gln-282) has been implicated in recognition of the exofacial ligand ATB-BMPA [79] and the whole motif is likely to constitute an important part of the exofacial binding site. Immediately preceding this sequence are residues QLS that are highly conserved in the transporters (GLUT 1, GLUT 3 and GLUT 4) which accept D-glucose with high affinity, but not in the transporters (GLUT 2 and GLUT 5) or the *Zymomonas molilis* [68] or trypanosome [73,80] transporters which accept D-fructose. The main difference between D-glucopyranose and D-fructofuranose is in the anomeric position at C-1 and C-2 respectively. The QLS residues may therefore be involved in docking the C-1 position of D-glucopyranose. Adjacent to the conserved regions in helix 7 are a series of conserved threonine and asparagine residues in helix 8. These may also constitute part of a hydrogen bonding channel allowing hexoses which are accepted at the exofacial site access to the inner binding site of the transporter. Release of sugars at the inner site may be controlled by conformational changes occurring in helices 10 and 11, where highly conserved tryptophan and proline residues are present. Molecular modelling and molecular dynamics studies suggest that prolines 383 and 385 are particularly important in facilitating an alternate opening and closing of the external site of these transporters [81] (Figure 3).

Ligand binding and labelling studies suggest some structural separation of external and internal binding sites. The bis-mannose labelling site has been mapped to helix 8 [83] and helix 9 [82].

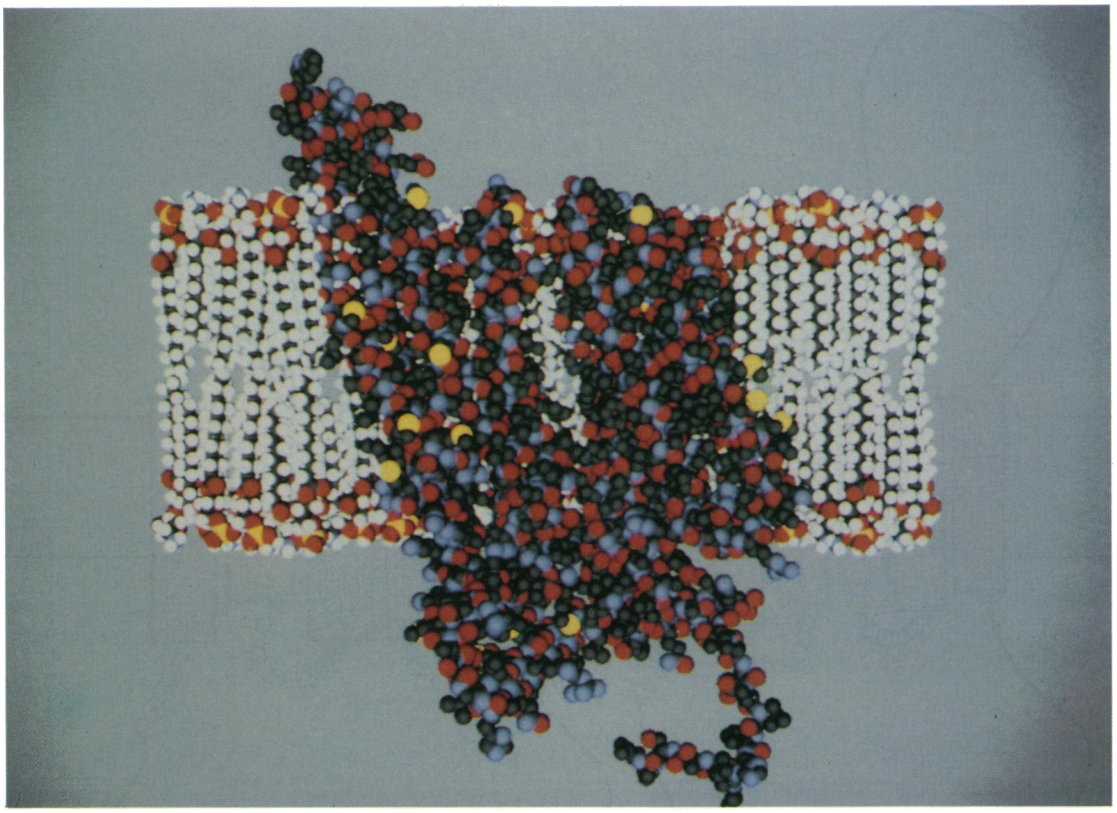

Figure 2 A hypothetical helix-packing arrangement for GLUT 1 (from [81])

The packing shown is that of a bilobular structure with six helices in each half of the protein. The shortness of the intracellular loops between helices restricts the allowable packing arrangements. Most of the protein is embedded in the membrane lipid but the N- and C-termini and the central loop connecting the two lobular domains are predicted to project into the cytoplasm.

However, the inside-specific ligand cytochalasin B labels a region between helices 10 and 11 [82–84], while the diterpine compound IAPS-forskolin labels helix 10 [85] and/or helix 9 [86].

Site-directed mutagenesis has already revealed some important features required for transport by GLUT 1. Truncation of the C-terminal region results in a mutated transporter that is locked in an inward-facing conformation that has low affinity for exofacial ligands such as the photolabel ATB-BMPA and results in a large reduction in sugar transport activity [87]. Mutation of Gln-282 in transmembrane helix 7 also results in the complete loss of exofacial binding of ATB-BMPA, but in this case the mutation only results in a 50% reduction in sugar transport activity and the binding of the inside-specific binding ligand cytochalasin B [79]. Mutation of the conserved Trp-412 in helix 11 of GLUT 1 and GLUT 4 results in reduced transport activity but no loss of binding of cytochalasin B [88] or IAPS-forskolin [86]. Mutation of Trp-388 of GLUT 1 expressed in CHO cells results in reduced transport and labelling with forskolin [86]. However, when this mutant was expressed in oocytes, it failed to insert correctly in the oocyte plasma membrane [89]. Consistent with the proposed important role of Pro-385 in the mode of operation of the transporter, it has been observed that mutating this Pro-385 to isoleucine in GLUT 1 markedly reduces glucose transport activity and ATB-BMPA labelling, but not cytochalasin B labelling [90].

Recently, Oka and colleagues have demonstrated that the replacement of the C-terminal domain of GLUT 1 with that of GLUT 2 renders the mutated transporter with transport kinetics more like those of GLUT 2 than GLUT 1, but cytochalasin B binding, which is normally lower for GLUT 2 than for GLUT 1, remained unaffected [91].

There is some debate as to whether GLUT 1 is oligomeric in its native state within membranes. Carruthers has suggested [92] that in the absence of reducing agents the GLUT 1 protein is tetrameric. It is suggested that oligomerization produces a form of the transporter in which the substrate, during transport in one subunit, induces a conformational coupling between subunits so that an external site in another subunit is re-exposed more rapidly than would occur in a non-coupled (monomeric) transporter. It remains to be determined definitively whether this oligomerization would confer any biological advantage to the transporter. However, Carruthers has speculated that the co-operative interaction between GLUT 1 monomers may result in a 2–8-fold increase in the catalytic activity of the transporter. The possibility that the catalytic activity of the transporter may be modulated *in vivo* by such a mechanism awaits exploration. Further evidence for oligomerization of GLUT 1 has been obtained from co-immunoprecipitation studies in 3T3-L1 adipocytes; while GLUT 1 oligomers were identified, no co-oligomerization of GLUT 1 and GLUT 4 proteins was identified in these cells [93].

INSULIN REGULATION OF GLUT 4 TRANSLOCATION

In 1980, Cushman and Wardzala [94], and independently Suzuki and Kono [95], first showed that in unstimulated (basal) adipose cells, glucose transporters (now known to be GLUT 4) were

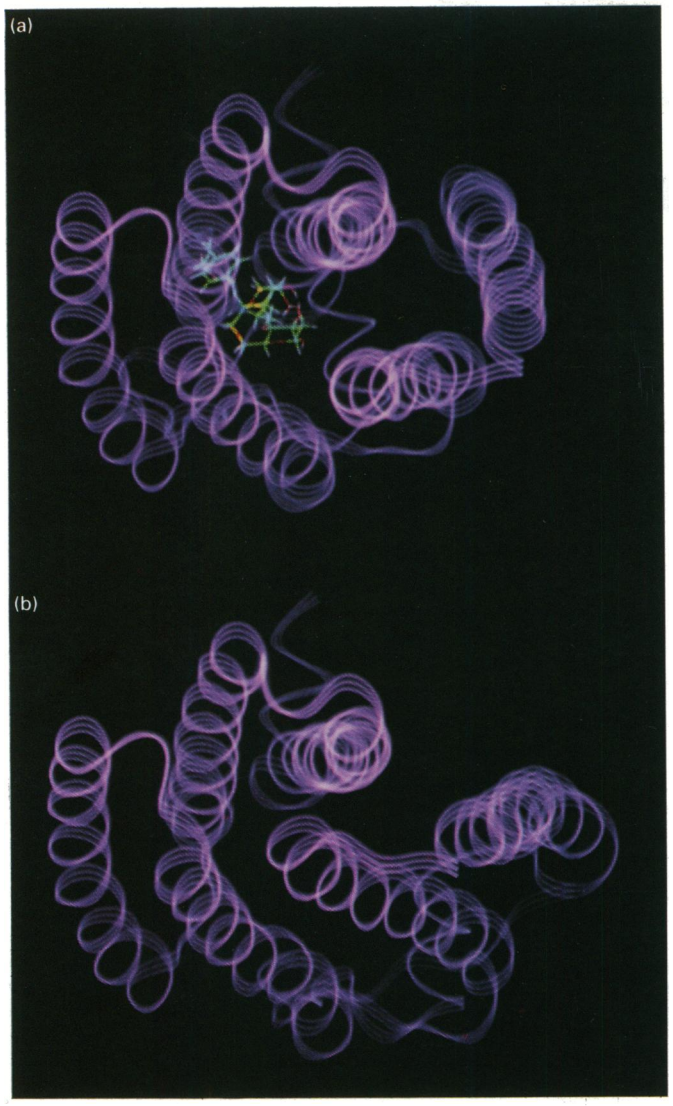

Figure 3 A putative mode of operation of the glucose transporters based on molecular dynamics simulations of GLUT 1 [81]

Highly conserved proline residues in GLUT 1 (residues 383 and 385 in helix 10) are predicted to act as a flexible region. Because of this flexibility, helices 11 and 12 can move relative to helices 7, 8 and 9, and open the outside glucose binding site and close the inner binding site (**a**). The C-terminal region of helix 12 is partly responsible for closing the inner site. Reversal of this helix flexing produces a closed site outside and an open site inside (**b**).

predominantly associated with a light microsome fraction of the cells, and that upon insulin stimulation, these transporters were recruited or translocated to the plasma membrane. An intracellular sequestration and an insulin-induced redistribution of these transporters to the plasma membrane was subsequently shown in other insulin-responsive tissues including brown adipose tissue [96], heart muscle [97,98], diaphragm muscle [99] and skeletal muscle [100–102].

A major obstacle that has hindered a resolution of the extent of the insulin-dependent subcellular redistribution of GLUT 4 has been the difficulty in obtaining pure membrane fractions. If plasma membranes from basal cells are cross-contaminated by light microsome membranes, which have high levels of GLUT 4, then this will lead to an underestimation of the extent of insulin-stimulated transporter redistribution.

Recent immunochemical techniques have circumvented the need to obtain subcellular membrane fractions [96–98]. These studies, involving the use of immunogold-tagged anti-GLUT 4 antibodies, have very clearly shown an intracellular location associated with tubulo-vesicular structures in the basal state, but a shift of GLUT 4 to the plasma membrane and early-endosome locations following insulin treatment. An additional approach to studying glucose transporter translocation (which also circumvents the requirement for obtaining membrane fractions to study insulin action) utilizes the cell-impermeant photolabel ATB-BMPA to selectively label the plasma membrane pool of transporters [51,103–106]. Because this label does not have access to the light-microsome-located transporters, it can be used to estimate both the extent and the rate of glucose transporter appearance in the plasma membrane following insulin-stimulation. Using this method, insulin was shown to increase the availability of GLUT 4 in the plasma membrane of rat adipocytes by 15–20-fold. In contrast, GLUT 1 labelling only increased by 3–5-fold.

The ATB-BMPA photolabel has been used to show that GLUT 4 is re-cycled to the light microsomes and back again to the plasma membrane even in the continuous presence of insulin [106]. GLUT 4 transporters (in insulin-stimulated rat adipose cells) were tracer-tagged with ATB-BMPA, and cells were then maintained either in the absence or in the continuous presence of insulin while subcellular trafficking was monitored. Under these conditions, it was found that the rate constant for endocytosis of the labelled transporters was similar in the presence and absence of insulin, but that re-exocytosis was markedly stimulated by insulin. Re-stimulation of cells in which the photolabelled transporter was internalized also showed that insulin increased the rate at which these transporters were transferred back (exocytosed) to the plasma membrane. Using the photolabel B3GL and an approach similar to that described for ATB-BMPA, Jhun et al. [107] suggested that insulin, in addition to increasing exocytosis, also reduced GLUT 4 endocytosis by 2.8-fold. Whatever the reason for these differences in the estimated endocytosis rates using ATB-BMPA or B3GL, it is clear that the major effect of insulin is to increase exocytosis.

GLUT 1 and GLUT 4 appear rapidly on the cell surface after insulin treatment of adipose cells, with half-times of about 2 min as detected by Western blotting and photolabelling. These half-times are about 1 min shorter that the half-time for the stimulation of transport, which increases with a half-time of 3 min. This lag between transporter appearance and participation in transport has been observed in both rat adipocytes [105,106,108] and 3T3-L1 adipocytes [109,110], and may occur because transporters, during the lag phase, are associated with trafficking proteins or may be present in occluded precursor states which do not fully expose transporters at the cell surface (Figure 4). The presence of these precursor states in the glucose transporter trafficking pathway may account for the observed disparities between the extent of translocation, as detected by Western blotting and photolabelling, and glucose transport activity under conditions of treatment with isoprenaline [111] and protein synthesis inhibitors [112]. Details of the trafficking intermediates involved in exocytosis have yet to be elucidated.

Some details are emerging of the endocytosis intermediates involved in removing cell-surface transporters. Endocytosis of transporters may occur via clathrin-coated pits and involve similar mechanisms to those which have been demonstrated for removal of cell-surface receptors. Slot et al. [96] in their immunocytochemical study of GLUT 4 in brown adipose tissue observed a GLUT 4–clathrin association in the plasma membrane and early endosomes. Similarly, Robinson et al. [113] observed that

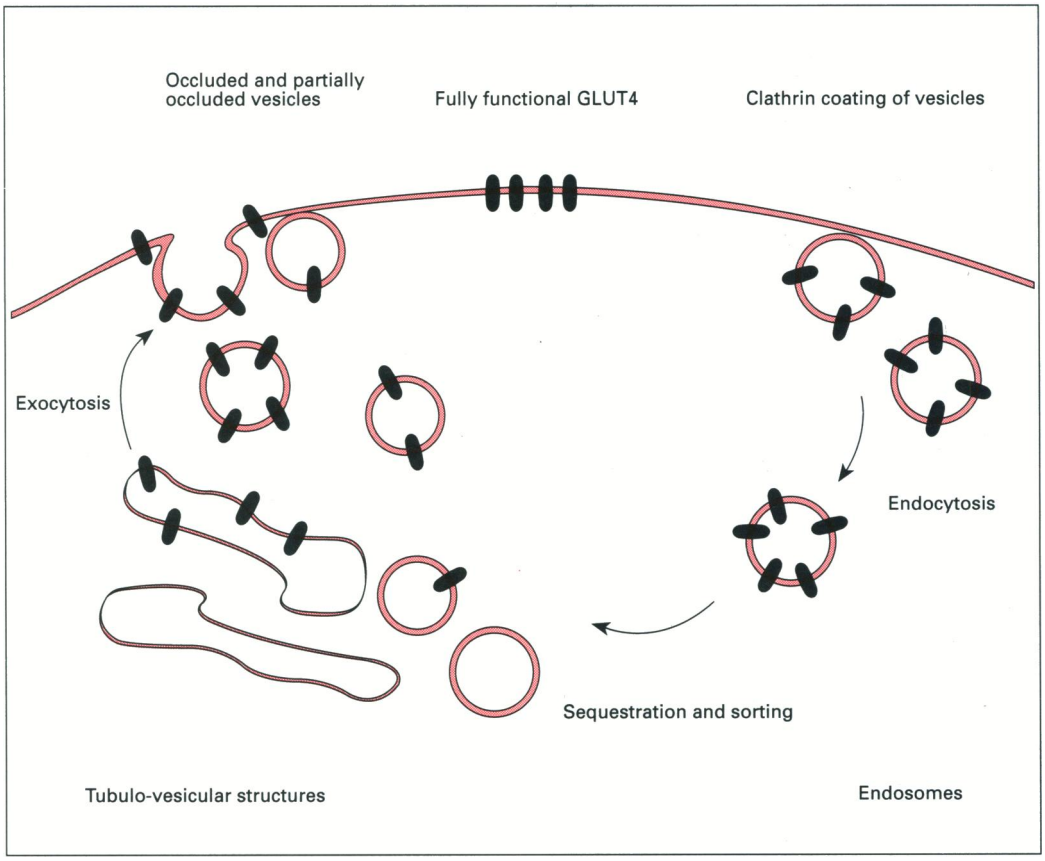

Figure 4 Insulin regulation of GLUT 4 translocation

GLUT 4 is predominantly present in intracellular tubulo-vesicular structures in the absence of insulin. Upon insulin stimulation, exocytosis is increased and GLUT 4 vesicles dock and fuse with the plasma membrane. Occluded and partially occluded structures in the plasma membrane may be responsible for slight discrepancies between the level of stimulation of glucose transport activity and GLUT 4 as detected by photolabelling (active and partially occluded forms) and by Western blotting (active, partially occluded and occluded forms). GLUT 4 is recycled in both the absence and the presence of insulin through clathrin-coated vesicles. Multiple GLUT 4 amino acid sequences responsible for targeting and sorting may be recognized by mechanisms present in the plasma membrane, the endosome recycling system and/or the tubulo-vesicular structures.

GLUT 4 was closely associated with flat clathrin lattices at the cell surface of 3T3-L1 cells.

The larger insulin stimulations of cell-surface availability of GLUT 4 (15–20-fold) compared with GLUT 1 (3–5-fold) are due to a lower rate of exocytosis of GLUT 4 in the basal state [114]. Although an intracellular sequestration of GLUT 1 has been demonstrated in several insulin-responsive cells, the proportion of the total cellular GLUT 1 which is maintained at the cell surface in the absence of insulin is much greater than for the GLUT 4 isoform. There is clearly some structurally distinct and unique property of GLUT 4 that results in its virtual absence from the plasma membrane in the basal state and this in turn results in this isoform responding acutely to insulin to produce the very large stimulations of glucose transport. There may be a unique targeting and sorting of the GLUT 4 protein, but not of GLUT 1, to a unique intracellular population of vesicles. These vesicles may have associated proteins that target the vesicles to a specific location in the tubulo-vesicular systems associated with the *trans* Golgi network. Evidence that GLUT 4 is located in different vesicles to the GLUT 1 isoform has been obtained in rat adipose cells [115] and skeletal muscle [116].

The GLUT 4 isoform has been shown to be sequestered to an intracellular pool when expressed in heterologous systems, including 3T3-L1 and NIH 3T3 fibroblasts [117,118], oocytes [119], CHO cells [120] and COS cells [121]. The implication from these studies is that GLUT 4 has a unique amino acid sequence or sequences within its primary structure which direct its targeting to an intracellular location. Piper et al. [122] have proposed that the N-terminal region of GLUT 4 is both necessary and sufficient for targeting of this isoform to intracellular pools. The N-terminal region of GLUT 4 is slightly longer than in the other mammalian isoforms, and this extension (MPSGFQQIGSED-GEPPQQ) may comprise an amino acid sequence that is recognized by intracellular targeting processes. However, other investigators have suggested that the N-terminal region of GLUT 4 is not necessary for intracellular targeting and have suggested that more central regions are required [120]. The problem of identifying targeting sequences is complex as, in addition to N-terminal and central regions of the transporter, the C-terminal 30 amino acids of GLUT 4 (ASSFRRTPSLLEQEVKPSTELE-YLGPDEND) have similarities to regions of the cation-sensitive mannose 6-phosphate receptor that have been implicated in intracellular targeting. At present, it is not clear whether multiple regions within the three-dimensional structure of GLUT 4 are folded to form a single unique targeting region that is recognized by a single chaperone protein that sorts GLUT 4 into an appropriate compartment. A perhaps more likely possibility is that several targeting regions within GLUT 4 are recognized by

separate chaperone proteins which are necessary at several steps in intracellular sorting (Figure 4). In their study of the targeting of the mannose 6-phosphate receptor, Johnson and Kornfeld [123] identified two separate motifs within the C-terminal tail that were required for sorting at the plasma membrane (YKYSKV) and to the *trans* Golgi network (LLHV). The C-terminal sequence of GLUT 4 therefore contains elements that can be considered as plasma membrane and *trans* Golgi network sorting signals. The investigation of the targeting of GLUT 4 to unique intracellular vesicles and the re-direction of these vesicles to the plasma membrane in response to insulin is currently a research area that is receiving intensive further study.

GLUCOSE TRANSPORTERS IN DISEASED STATES

Is GLUT 2 a component of the glucose-sensing apparatus of the β-cell?

Higher mammals can sense and respond to elevated blood sugar levels by secreting insulin within minutes. The glucose transporter expressed in β-cells has the same primary sequence as that expressed in the liver, i.e. it is GLUT 2 [124]. Immunolocalization has demonstrated that the protein is expressed predominantly in the microvillar portion of the plasma membrane, facing the adjacent endocrine cells, and that the protein is not expressed in the α- or δ-cells [35]. Some circumstantial evidence supports the proposal of a potential role for GLUT 2 in glucose sensing. Unger's laboratory showed that GLUT 2 expression in rat β-cells could be down-regulated by chronic hyperinsulinaemia. The islets from these animals were essentially devoid of GLUT 2 mRNA, and the glucose transport characteristics of the β-cells showed that the transport K_m was some 7-fold lower, indicative of a switch in the isoform of transporter expressed. The new K_m value, about 2.5 mM, is roughly half the typical fasting blood glucose concentration [125]. This result implies that the loss of GLUT 2 function would render β-cells unable to sense and respond to changes in circulating blood glucose levels above about 5 mM, and hence postprandial hyperglycaemia would not be corrected. It is notable that, as well as a reduction in the K_m, a significant reduction in the $V_{max.}$ for transport is also observed in islets from hyperinsulinaemic rats, and thus the overall capacity of the islets to transport glucose is markedly reduced [126]. Evidence for a possible role of GLUT 2 in glucose sensing has been suggested by investigators studying patterns of GLUT 2 expression in diabetes (see below).

However, it should be pointed out that two recent studies have seriously questioned the importance of GLUT 2 in the β-cell glucose-sensing apparatus [127,128]. Most significantly, it has recently been demonstrated that the glucose utilization rate of freshly isolated islets is 100-fold lower than the extent of glucose transport [127]. In freshly isolated islets, it would seem clear that the rate of glucose transport would have little consequence on the rate of glycolysis, and thus a role for GLUT 2 in glucose sensing would appear unlikely. Moreover, in a parallel study, it has been demonstrated that first-phase glucose-stimulated insulin release is unchanged or even enhanced in islets cells cultured in glucose, culture conditions which markedly reduce GLUT 2 levels in the β-cell. Further evidence against a role for GLUT 2 in glucose sensing has emerged from studies of a transgenic mouse engineered to express a transforming ras protein in the β-cells. Surprisingly, these animals have been shown to be completely normal with respect to the time course and extent of insulin secretion in response to a glucose bolus, but interestingly, the β-cells of these animals do not express GLUT 2 [128]. These observations are difficult to reconcile with the results alluded to above, which suggest that GLUT 2 expression appears to modulate glucose-induced insulin secretion, and this dichotomy awaits resolution.

GLUT 2 expression changes in type I and type II diabetes

Insulin-dependent, or type I, diabetes mellitus is an autoimmune disease of β-cells. It affects predominantly children and younger adults, and is correlated with an inherited susceptibility linked to a class II major histocompatibility molecule [129]. The onset of type I diabetes occurs gradually, but a clinical manifestation of the metabolic abnormality does not occur until about 80% of the β-cells have been destroyed. During the pre-diabetic phase, the only identified symptom is the blunting of the first-phase insulin response to intravenous glucose. During this development period, several antibodies to β-cell proteins will be present in the serum of patients. These antibodies include antibodies to glutamic acid decarboxylase and heat-shock protein 65, as well as to other unidentified β-cell proteins, insulin auto-antibodies and β-cell surface antibodies [130–132].

One interesting and potentially important observation has come from the demonstration by Johnson et al. that immunoglobulins from newly diagnosed type I diabetics can affect GLUT 2-mediated glucose transport in normal islets [133]. Both the K_m and $V_{max.}$ of GLUT 2-mediated glucose transport in rat islets were reduced by IgG from diabetic patients compared to control patients. These and other data from Unger's laboratory suggest that GLUT 2, or a protein which modulates GLUT 2 activity, is a target for islet cell antibodies, but definitive evidence of a direct immunological reaction with GLUT 2 has yet to be demonstrated, and this inhibition of GLUT 2 activity by serum IgG from diabetics could not be demonstrated in oocytes expressing GLUT 2 [134].

It is of note that the amount of GLUT 2 protein is also reduced in the β-cells of animals undergoing autoimmune destruction. Thus, studies of the BB rat model of autoimmune diabetes have shown that less than half of the surviving β-cells express GLUT 2. This reduction of GLUT 2 levels is further magnified since the total number of β-cells is only 20% of that from a control animal, resulting in a 90% reduction in the number of GLUT 2-positive β-cells [135].

Type II (non-insulin-dependent) diabetes occurs in mature adults and is associated with abnormal insulin secretion and severe peripheral insulin resistance (insulin-resistant glucose transport is described in the next section). Evidence for a defect in β-cell function related to changes in GLUT 2 in type II diabetes has been provided by an analysis of the partially inbred glucose-intolerant Zucker fatty (*fa/fa*) rat [136]. All male rats become obese and develop overt type II diabetes between 7 and 9 weeks of age, whereas neither the *fa/fa* females, which are as obese as the *fa/fa* males, nor the lean male and female heterozygotes develop hyperglycaemia. Insulin secretion in the perfused pancreas from diabetic male rats responds to 10 mM arginine, but not to 20 mM glucose. In contrast, the age-matched female littermates respond to both [136]. Using immunofluorescence, Orci et al. showed that in male rats at the pre-diabetic stage GLUT 2 expression was normal in the β-cells, but upon development of overt diabetes GLUT 2 expression was essentially undetectable [137]. Similar decreases in GLUT 2 mRNA levels were recorded. This decrease in GLUT 2 expression was paralleled by a profound reduction in high-K_m glucose transport into isolated islets [137]. Two subsequent studies have demonstrated that the loss of immunoreactive GLUT 2 is not secondary to the onset of hyperglycaemia, thus establishing a potential link between the reduction of GLUT 2 expression, the loss of glucose-

stimulated insulin secretion and the resulting steady-state hyperglycaemia.

GLUT 1 and GLUT 4 in type II diabetes and insulin resistance

Type II diabetes is associated with severe peripheral insulin resistance. Insulin resistance is also linked with other syndromes and it is considered to be a major contributing factor to hypertension, atherosclerosis and coronary heart failure. It has been suggested by Dowse and Zimmet [138] that, with modern life-style changes, insulin resistance and its consequences can be considered as a major health epidemic.

Insulin resistance is characterized by a failure of insulin to result in efficient glucose disposal, and in particular by a failure of insulin to produce its normal increase in glucose transport in target tissues. The main site of glucose disposal is muscle and this tissue is therefore considered to be most important in terms of the site of insulin resistance. Adipose tissue accounts for only 5–20% of glucose disposal. However, much of the review of the experimental work on insulin-resistant glucose transport that is described here concerns studies on adipose tissue, because many of the mechanistic studies on this problem have been easier to address in this tissue. In muscle, mechanistic studies are rendered more difficult because of the inherent problems associated with preparing subcellular membrane fractions to assess the localization and translocation of the GLUT 4.

As described earlier, it is the propensity of GLUT 4 to become sequestered within the cells of non-stimulated adipose and muscle tissue that renders these tissues uniquely sensitive to insulin. GLUT 1 is not sequestered as efficiently as GLUT 4 and consequently only small increases in the recruitment of this isoform to the plasma membrane occur. A loss of cellular GLUT 4 could lead to insulin resistance, but a loss of the sequestration process for GLUT 4 and/or a decrease in its translocation to the plasma membrane may also contribute to impairment in insulin-responsiveness of glucose transport.

A depleted intracellular pool of glucose transporters in adipose tissue from obese and type II diabetes patients has been observed [139–141]. Similar changes can be induced by streptozotocin treatment of rats and in fasting rats, and these latter effects have been specifically attributed to GLUT 4 depletion. The mRNA and protein are decreased but, in the case of starved rats, the GLUT 4 level can be restored by re-feeding [142]. In rat adipocytes which are maintained in culture for 24 h the development of poor insulin-responsiveness of glucose transport is due to a decrease in GLUT 4 and to a shift in the ratio of GLUT 4 to GLUT 1 [143]. In freshly isolated cells this ratio is 9:1, but it is reduced to 3:1 in the cells maintained in culture for 24 h [143]. Thus there is a shift away from the acutely insulin-sensitive isoform GLUT 4 to the poorly sequestered and insulin-responsive isoform GLUT 1. A similar shift towards a greater contribution of GLUT 1 to the transport activity also occurs in adipocytes from obese rats. The shift in the GLUT 1/GLUT 4 ratio may be associated with a de-differentiation of the adipose cells. The insulin-resistance syndrome in general may be a consequence of de-differentiation of insulin target tissues. Consistent with this possibility, Block et al. [144] have shown that a shift in the GLUT 4/GLUT 1 ratio also occurs in muscle cells that are denervated, begin to de-differentiate and become insulin-resistant.

Cellular depletion of mRNA and protein cannot always account for the observed deficiency in glucose transport activity in obese and type II diabetes patients [145–149]. Similarly, in the *db/db* mouse model of insulin resistance there are no changes in the total cellular content of GLUT 4 [147]. Pedersen et al. [147] have found that, in muscle from type II diabetes patients, there are no significant changes in GLUT 4 mRNA or protein. However, Dohm et al. [148] report that small (18%) decreases in GLUT 4 are observed in type II diabetes patients. Recent findings [150–152] have led to the suggestion that insulin resistance in glucose transport may be due to defective translocation of GLUT 4 in muscle.

An insulin resistance of glucose transport that is induced by chronic insulin treatment has been demonstrated to occur in human adipose cells. In the adipose cells from obese and type II diabetes patients, prolonged insulin treatment exacerbates the insulin resistance [153]. Several *in vitro* animal models of the insulin resistance that follows chronic insulin treatment have been developed. Garvey et al. [154] and Traxinger and Marshall [155] have shown that insulin resistance in glucose transport that is induced by chronic insulin treatment of primary cultured rat adipocytes is neither at the insulin receptor level nor due to a depleted intracellular pool of transporters. If rat adipose cells are maintained in the continuous presence of insulin during the culture period then GLUT 4 is down-regulated from the cell surface, but the total cellular level of this transporter does not fall below that found when cells are cultured without insulin [143]. The down-regulation of GLUT 4 from the cell surface is associated with a marked decrease in the ability of the cells to respond to a further challenge with insulin. In chronically insulin-treated adipose cells, re-challenging with insulin only increased transport to 30% of the normal response of cells cultured without insulin [143]. It is unclear whether insulin resistance in type II diabetes could be causally related to chronic insulin. Hyperinsulinaemia is always present in the early stages of type II diabetes but this could be a consequence of the resistance. Thus, more insulin may be secreted to compensate for the ineffectiveness of circulating insulin to produce its normal stimulation of glucose disposal.

Several drugs, including sulphonylureas and biguanides, which are used in the treatment of type II diabetes have been shown to potentiate insulin's action on glucose transport [156–159]. The biguanide metformin alleviates the insulin resistance found in cultured rat adipocytes which have been chronically treated with insulin. Using the photolabel ATB-BMPA it has been shown that, in this system, metformin treatment with chronic insulin treatment prevents the down-regulation of cell surface GLUT 4 [143]. Metformin has also been shown to enhance glucose transport activity in L6 muscle cell lines [158] and in skeletal muscle [159].

FUTURE DIRECTIONS

Transporter structure and kinetics

A major area of current and future investigation involves the use of molecular biology techniques to elucidate structural domains of the glucose transporters that are involved in substrate recognition and transport catalysis. It is likely that from further mutagenesis and labelling studies the relationship between the structure of the glucose transporters and their function will gradually emerge. Particular questions that can be addressed using molecular biology techniques are the identification of amino acids and the structural domains that confer the unique functional and kinetic properties to each of the glucose transporter isoforms, and the identification of the targeting signals in the protein sequence that are necessary for directing each of the isoforms to a specific subcellular location.

Diabetes

The investigation of the role of glucose transporters in diabetes is an area that is likely to produce considerable future advances. In particular, the investigation of the role of GLUT 2 in insulin secretion and the role of GLUT 4 in peripheral insulin action will be greatly facilitated by the detailed knowledge of the structure and function of these isoforms that will emerge from mutagenesis and chimera studies. Given the important role of GLUT 2 in the regulation of whole-body glucose homeostasis, the identification of the factor/factors which initiate the loss of islet cell GLUT 2 would represent a significant advance in our understanding of the development and control of the symptoms of diabetes. Future studies will also be aimed at determining the factors which regulate the expression of GLUT 4 in type II diabetes. Perhaps more importantly, the issue of GLUT 4 expression and regulation in muscle from type II diabetics needs to be resolved. Much emphasis will in future be placed on elucidating the signalling route between the insulin receptor and the GLUT 4 translocation pathway.

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